Visual Evoked Cortical Potentials in the Cat after Retinal Modifications Induced by Intraocular Kainic Acid

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SIGÜENZA, J. A. AND P. GOMEZ-RAMOS. *Visual evoked cortical potentials in the cat after retinal modifications induced by intraocular kainic acid.* PHARMACOL BIOCHEM BEHAV 20(1) 79–83, 1984.—Intraocular injections of kainic acid (KA) induce changes in visual-evoked cortical potentials in the cat at the level of the primary and secondary wave complexes. These changes are correlated with histological findings that show degeneration of cells in the inner nuclear layer and ganglion cell layer. These results indicate that despite the cell death produced by KA, certain retinal circuits continue to function. The fact that the first modifications induced by KA are observed at the level of the primary complex agrees well with the proposed retinal origin of this complex. The modifications of the secondary complex may imply a different transmission and/or processing of visual information at the central level.

Cortical evoked potential   Kainic acid   Retinal toxicity

KAINIC acid (KA) is a structural analogue of glutamate which has both excitatory [10,15] and toxic [13] actions on the central nervous system. Intraocular injections of KA produce extensive degeneration of neurons in the retina of fish [17], birds [14] and mammals [8]. Degeneration of KA-sensitive cells in the retina must disrupt the neuronal circuitry resulting in the visual changes that can be studied by electrophysiological methods [7].

Visual-evoked cortical potentials (VECP) have begun to be used in clinical practise to assess brain function or dysfunction [3]. The establishment of animal models in which the modifications of VECP induced by specific lesions or by the use of toxic substances can be studied would help to clarify our understanding of the nature of VECP and how they are modified by experimental or pathological alterations in the cat retina induced by intraocular injections of KA and the concomitant modifications of VECP.

METHOD

VECP were recorded in seven adult male cats. After opening the right frontal sinus of the cat a small light-emitting diode (LED) which was used as the source of visual stimuli was placed in direct contact with the eyeball. LED firing was controlled by a Grass S-88 stimulator adjusted at a frequency of 1 Hz and an intensity of 45 mA. Further details of the procedure and specifications can be found in a previous paper [16].

For recording two stainless-steel electrodes were implanted over the left primary visual cortex (21 and 23 mm from bregma and 1 and 3 mm laterally from midline). LED and electrodes were attached to the skull with acrylic cement. During the recording period the animals were kept in a soundproof chamber which was adapted to scotopic conditions. The animals were connected to an electroencephalograph machine to record the evoked responses. In all cases one hundred single responses were averaged in a Didac-4000 averaging computer.

Intraocular Injections

Under deep ether anesthesia the right eye (the one which had the implanted LED) was injected with 100 μl of a freshly made aqueous solution (pH: 7.2) containing 100–200 nmoles of KA (Sigma) and colored with bromophenol blue to allow assessment of the homogeneity of distribution of the solution.

Recordings

Control recordings were obtained before the intraocular injections of KA. Experimental recordings began 45 minutes after the intraocular injections in order to avoid the effect of ether anesthesia on the waveform of the VECP. Successive recordings were obtained every hour on this first day and were repeated every day for the rest of the testing period which lasted one week.

Histological Procedure

Most of the animals were killed by an overdose of anesthesia at the end of the testing period but in some cases shorter survival times were used to study the temporal course of the KA effect on the histology of the retina. The retinas were fixed by immersion in a solution of 3% glutaraldehyde in 0.1 M phosphate buffer plus 5% sucrose, postfixed in osmium tetroxide, dehydrated, and embedded in Epon 812. The sampling procedure consisted of a rectangle of ap-

TABLE 1

Latencies

		a	b	c	d	e	f	g
Basal	mean	44.28	69.57	81.28	99.28	129.93	179.64	254.36
	SD	19.67	17.83	14.25	14.70	28.13	38.36	56.90
		a		C		e	f	g
2 hr postKA	mean	47.32		83.09		134.00	196.66	270.26
	SD	15.12		21.79		18.52	10.41	26.46
4 hr postKA	mean	43.33		84.23		146.66	235.27	278.33
	SD	12.89		25.72		17.64	37.64	70.05
7 days postKA	mean	40.62		85.12		146.87	251.14	290.07
	SD	14.99		21.15		17.72	47.07	44.53

Amplitudes

		a–b	b–c	c–d	d–e	e–f	f–g
Basal	mean	14.75	12.25	12.38	30.38	34.85	27.50
	SD	4.43	7.04	5.99	19.19	17.25	24.61
		a–C			C–e	e–f	f–g
2 hr postKA	mean	29.33			44.13	37.66	34.26
	SD	3.52			13.22	15.14	15.13
4 hr postKA	mean	30.33			51.33	80.33	35.66
	SD	4.08			18.50	14.74	17.02
7 days postKA	mean	27.62			63.80	112.00	42.60
	SD	11.37			20.80	52.57	16.93

This table shows a tabulation of mean peak-to-peak amplitude and latency values with their standard deviations (SD) for the Basal, 2 hours postKA, 4 hours postKA and 7 days postKA recordings.

proximately 6 mm long by 3 mm wide taken from the central tapetal area about 5 mm up from the optic nerve head. Transversal sections 1 μm thick were stained with toluidine blue.

Statistical Analysis

A tabulation of mean peak-to-peak amplitude and latency values with their standard deviations was done for the basal recordings and for the post-KA recordings (2 hr, 4 hr and 7 days) as show in Table 1.

Statistical comparisons (*t*-test) were done between each two recordings for each latency and amplitude when possible, for instance latencies of "b," "c" and "d" waves and

"a"–"b," "b"–"c," "c"–"d" and "d"–"e" amplitudes only exist in the basal recordings and statistical comparisons with the post-KA recordings were impossible. However statistical comparisons were also done between each two post-KA recordings for the latencies and amplitudes of the new VECP components, for instance latency of "C" wave and "a"–"C" and "C"–"e" amplitudes.

RESULTS

The characteristics of a control VECP obtained by LED stimulation are shown in Fig. 1B. Two wave complexes can be distinguished, the first of which (primary complex) was assigned the letters "a," "b," "c" and "d" while the sec-
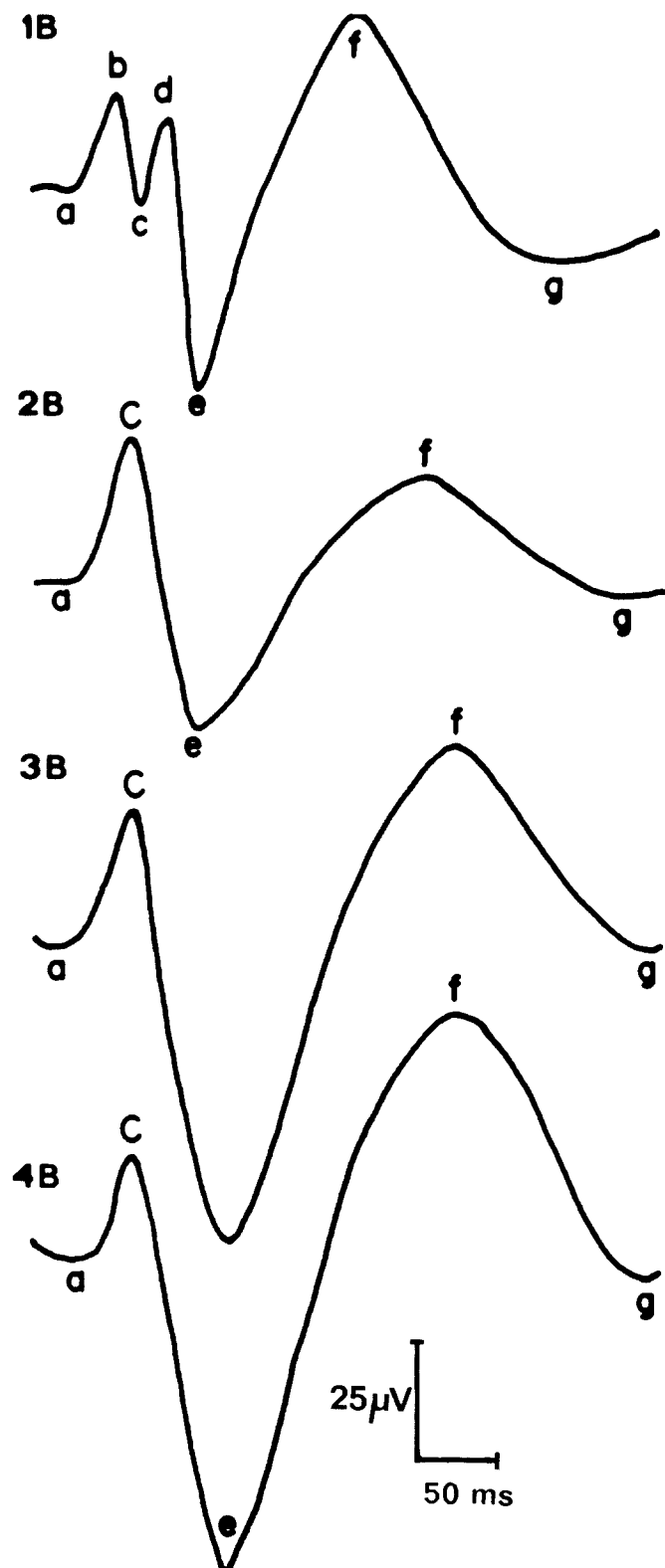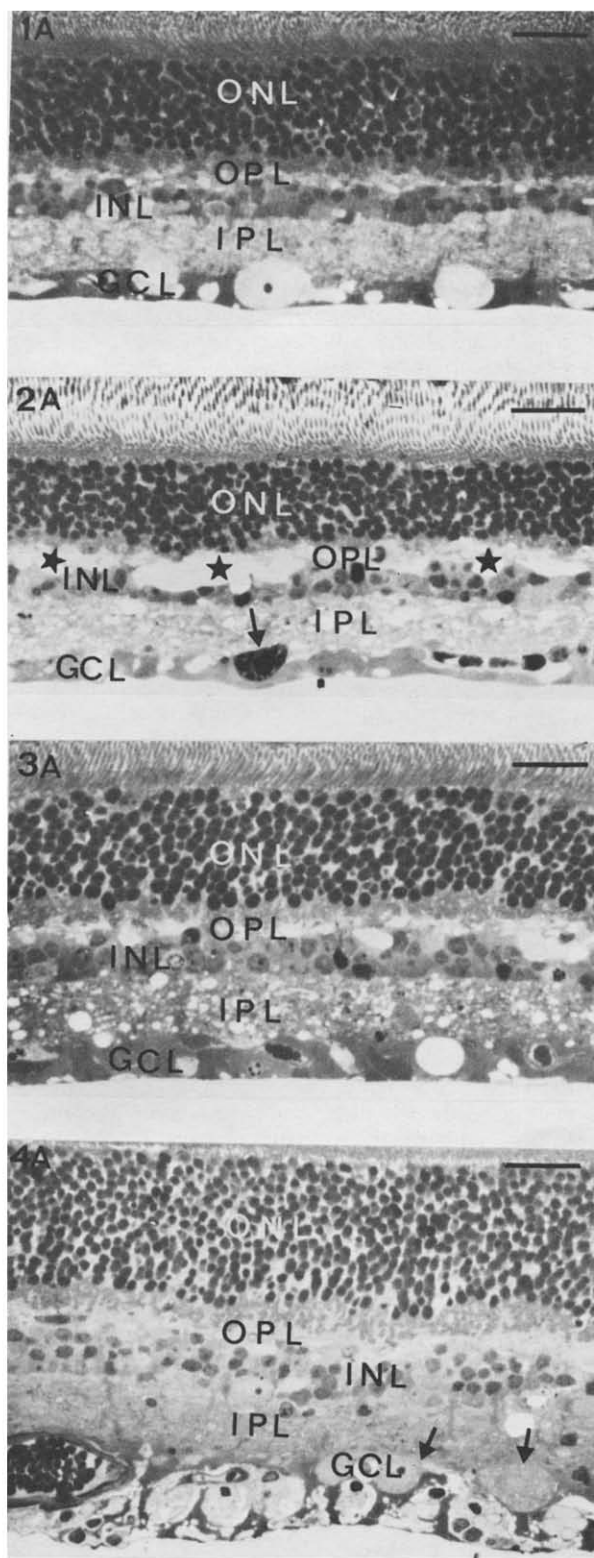
FIGS. 1–4

ond (secondary complex) was assigned the letters "e," "f" and "g." In all cases, forty-five minutes after the KA injection, the first experimental recordings showed modifications of VECP at the level of the primary complex. The "a" waves remain constant but the "b," "c" and "d" waves disappeared and instead a new wave named "C" appeared. This modification in the primary complex (Fig. 2B) remains constant up to the end of the week (Figs. 3B and 4B). The secondary complex starts to change from 2 hours post-injection. After this time the wave pattern also showed an increment in the "e"–"f" amplitude. This increment reaches a significant level by 4 hours post-injection (Fig. 3B) and the pattern continues to be altered until the end of the experiment a week later. The statistical comparisons from the data of Table 1 showed that only significant changes were present at the level of the latency of the "f" wave in the comparison between the basal recording and the 4-hours and 7-days post-KA recordings. At the level of the amplitudes the only significant changes were present at the level of the "e"–"f" amplitude in the comparisons between the basal recording and the 4-hours and 7-days post-KA recordings, and in the comparisons between the 2-hours post-KA recording and the 4-hours and 7-days post-KA recordings.

Histological alterations were already present in the inner retina two hours after the intraocular injections of KA (compare Fig. 1A and 2A). In the inner nuclear layer (INL) cells which could be horizontal cells according to their position and appearance were highly swollen, whereas others located in the inner border of the INL which seem to be amacrine cells showed signs of pyknosis. In the inner plexiform layer (IPL) there were abundant large vacuoles which were also found among the cells of the ganglion cell layer (GCL) (Fig. 2A). In the GCL some cells showed degenerative changes characterized by darkening of the whole cell clumping of the chromatin. In a few instances a highly swollen cell was present in this layer. The presence of swollen cells had decreased by 4 hours but at this time there were still abundant dark-degenerating cells in the INL and GCL (Fig. 3A). A week after the intraocular injection of KA the retina appeared normal except for the nerve cell layer in which there was an abundant infiltrate of small, dark-stained cells resembling glial cells (Fig. 4A). The INL seems to contain fewer cells and the same was true for the GCL, but the IPL had recovered its compact structure. An increased vascularization was evident in the inner retina (Fig. 4A).

At the behavioral level the animals appeared normal and showed light reflex in both eyes. A few days after the injections of KA it was observed that the pupillary diameter of the KA-injected eye was smaller than the one in the control eye. Also, most of the animals squinted with the KA-injected eye when they were in bright light.

DISCUSSION

As it has been described in our results one of the main findings at the electrophysiological level is the disappearance of the "b," "c" and "d" waves of the primary complex and the concomitant appearance of the new wave "C." This find-

ing can be interpretated more as a wave fusion, in which "C" would be the resultant wave, than a modified "b" or "d" component as show Table 1 and the statistical treatment. Since it has been shown that the primary complex is determined by the conditions of the stimulus [2,11] and thus related to the retina, the present results could be considered as a confirmation of the retinal origin of the primary VECP complex, because it is in the primary complex that the earliest modifications of VECP are observed. This wave fusion in the primary complex may represent a change in the temporal pattern of afferent response.

The increased amplitude of the secondary VECP complex and the augmented response to light observed behaviorally are consistent with a lower light threshold, although other explanations are also possible at this stage. Since the secondary complex originates from an endogenous elaboration of the brain [1, 2, 12], the observed changes in this secondary complex of the VECP may also imply a different transmission of visual information at the central level and/or a different modification of this information.

The histological data show a clear effect of KA on retinal cells in the INL and GCL. In the INL highly swollen cells in the outer border resemble horizontal cells, and dark-degenerating cells in the inner portion are believed to be amacrine cells. In the GCL the degenerating cells could be ganglion cells, displaced amacrine cells or both. Displaced amacrine cells degenerate after KA in the chicken [4], and a population of microneurons is tentatively identified as displaced amacrine cells in the cat [9]. Ganglion cells also seem to be sensitive to KA in the rat since axoplasmic transport in the optic nerve is affected after intraocular KA [5] and in summary a variety of cells are affected by KA in the retina and some of these cells are certainly killed. The death of some ganglion cells as well as the death of other retinal cells which influence the surviving ganglion cells should alter the output of retinal information as reflected in the modification of the primary VECP complex already discussed.

Despite the cell loss induced by KA certain retinal connections are still functioning one week after the injection of KA as the presence of VECP and light reflex indicates. Furthermore, a week after the KA the retinal ganglion cells showed a very high response to acetylcholine [6] indicating that the surviving ganglion cells are capable of processing information. Although little can be stated in the present study about which retinal circuits survive KA treatment, preservation of the rod neural pathway has been suggested in the goldfish [17].

In summary, the present study shows the temporal modifications in VECP when retina is damaged by the highly toxic substance kainic acid. It would be interesting to see whether a similar pattern of VECP modifications is present in any of the many cases in which human retinal degeneration is observed.

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